

iGEM EPFL: CpxR-IFP Stress Experiment

Materials

- Biliverdin hydrochloride 25 mM (Dissolved in 100% DMSO) [30891 SIGMA]
- L-(+)-Arabinose 20% (Dissolved in bi-distilled water) [A3256 SIGMA]
- LB Chloramphenicol (25 µg/mL)
- 250 ml Erlenmeyer
- 14 ml Polystyrene Round-Bottom Tubes

Procedure

1. Grow a starter culture overnight in 3ml of LB with the appropriate antibiotic
2. Transfer 40 µl of the starter culture in 25 (ml) of LB with the appropriate antibiotic and grow in a 250 (ml) Erlenmeyer at 37°C with shaking at 180 rpm until reaching an OD₆₀₀ of 0.6
3. Transfer cells in 8% arabinose (1.2 ml) with 3(ml) of total volume [14 ml Polystyrene Round-Bottom Tubes] and grow for 2 hours at 37°C with shaking at 180 rpm
4. Thaw biliverdin for 5-10 min while protecting from light. Add 1 µl of biliverdin hydrochloride 25 mM in the dark (no direct light) to get a final concentration of 8.33 µM. Grow for 3 hours at 37°C with shaking at 180 rpm
 - **IMPORTANT:** Pipet Up and Down thoroughly (about 30 times) until biliverdin is solubilized!
5. Swirl the tubes 5-6 times to get the remnant biliverdin in the solution, centrifuge at 550 x g (2800 rpm on medium centrifuge) for 10 min, and wash subsequently in 320 µl of PBS (except for KOH stress)
6. Calculate the volume of cells needed for 100 µl total volume and the appropriate stress. Transfer into 96 well plate for plate reading.
7. Read about 20 min without stress and then add stress and continue reading for 2 hours.

Annexe:

Read	Parameters
Fluorescence 100%	Emission: 640 nm Excitation: 708 nm
Fluorescence 70%	Emission: 640 nm Excitation: 708 nm
OD ₆₀₀	Absorbance: 600 nm

